Biochimica et Biophysica Acta 1852 (2015) 651–657

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Myasthenia gravis and related disorders: Pathology and molecular pathogenesis ☆

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ARTICLE INFO

Article history:
Received 1 July 2014
Received in revised form 20 November 2014
Accepted 29 November 2014
Available online 6 December 2014

Keywords:
Neuromuscular junction
Myasthenia gravis
Lambert–Eaton myasthenic syndrome
Botulism
Organophosphate poisoning
Congenital myasthenic syndrome

ABSTRACT

Disorders affecting the presynaptic, synaptic, and postsynaptic portions of the neuromuscular junction arise from various mechanisms in children and adults, including acquired autoimmune or toxic processes as well as genetic mutations. Disorders include autoimmune myasthenia gravis associated with acetylcholine receptor, muscle specific kinase or Lrp4 antibodies, Lambert–Eaton myasthenic syndrome, nerve terminal hyperexcitability syndromes, Guillain Barré syndrome, botulism, organophosphate poisoning and a number of congenital myasthenic syndromes. This review focuses on the various molecular and pathophysiological mechanisms of these disorders, characterization of which has been crucial to the development of treatment strategies specific for each pathogenic mechanism. In the future, further understanding of the underlying processes may lead to more effective and targeted therapies of these disorders. This article is part of a Special Issue entitled: Neuromuscular Diseases: Pathology and Molecular Pathogenesis.

1. Introduction

Diseases of the neuromuscular junction (NMJ) (Table 1) produce weakness which generally varies with repeated synaptic firing, i.e. sustained or repeated muscle contraction [1–3]. The "true" myasthenias are NMJ disorders in which weakness worsens with sustained muscle contraction or work and improves with rest. Analysis of these diseases has determined that they primarily involve components of the postsynaptic portion of the NMJ. The other disorders of the NMJ result in weakness that either improves or remains unchanged with exercise. The latter group is associated with dysfunction of the presynaptic apparatus or of the components of the synaptic cleft. The clinical characteristics of the "fatiguing" weakness in an individual patient with one of the "true" myasthenias can be confirmed electrophysiologically by decrementing compound muscle action potentials (CMAPs) in response to slow rates of motor nerve stimulation (2–3 Hz), in association with normal amplitudes of the responses to single nerve stimuli. In contrast, most of the presynaptic NMJ disorders are associated with reduced amplitudes of the CMAPs in response to single motor nerve stimuli but with increased amplitudes following rapid stimulation of the nerve.

The majority of patients with NMJ dysfunction have either myasthenia gravis (MG) or Lambert–Eaton myasthenic syndrome (LEMS) [4,5]. Remarkably, both diseases are autoimmune in nature and, moreover, each results from a T cell-directed antibody (Ab)-mediated attack on ion channels that are crucial for neuromuscular transmission (NMTx) via the NMJ. For MG, the target of the auto-Ab attack is the nicotinic acetylcholine receptor (AChR) in the postsynaptic membrane, whereas for LEMS the target is the P/Q-type voltage-gated calcium channel (VGCC) located in the presynaptic motor nerve terminal membrane. In a third disorder, autoimmune neuromyotonia, Ab-mediated attack on the nerve terminal (rectifying) voltage-gated potassium channel results in spontaneous firing of the synapse [6]. In Guillain Barré syndrome Abs directed against gangliosides GM1 and GQ1b have been shown to affect NMTx at the NMJ in addition to demyelinating and axonal nerve damage. The reasons for the particular susceptibility of the NMJ to autoimmune attack have not yet been elucidated. Nonimmune diseases also lead to disordered NMTx. Organophosphate poisoning results in blockade of the muscle acetylcholinesterase, leading to NMJ dysfunction from excessive neurotransmitter activity. In botulism, the various toxins bind to and hydrolyze individual intracellular presynaptic proteins involved in docking and release of (acetylcholine (ACh)-containing vesicles. In addition, over the last twenty years or so, a group of genetically determined congenital myasthenias have been identified and studied. Each congenital myasthenic syndrome (CMS) results from a mutation in a protein important to NMTx. The mutations have been classified as to whether they involve presynaptic, synaptic cleft or postsynaptic proteins [7].

2. Review of NMJ structure and function

The NMJ is the most highly studied neural synapse—primarily because of its location in the peripheral nervous system isolated from

☆ This article is part of a Special Issue entitled: Neuromuscular Diseases: Pathology and Molecular Pathogenesis.

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http://dx.doi.org/10.1016/j.bbadis.2014.11.022
0925-4439/

Table 1
Features of various neuromuscular junction disorders. NMJ = neuromuscular junction, AChR = acetylcholine receptor, MuSK = muscle specific receptor tyrosine kinase, Lrp4 = lipoprotein receptor-related protein 4, LEMS = Lambert-Eaton myasthenic syndrome, VGCC = voltage gated calcium channel, VGKC = voltage gated potassium channel, CMS = congenital myasthenic syndrome.

NMJ disorder	Synaptic location	Autoantibody	Protein(s) involved	Thymic abnormalities	Age at onset (years)	Female : male ratio
Autoimmune MG						
AChR MG	Postsynaptic	Nicotinic AChR	–	Lymphoid hyperplasia and thymoma	20–30 (women) and > 50 (men)	1:2 (3:1 in juvenile MG)
MuSK-MG	Postsynaptic	MuSK	–	–	Variable	8.5:1
Lrp4-MG	Postsynaptic	Lrp4	–	Unknown	> 40 (limited data)	2.5:1
LEMS	Presynaptic	P/Q type VGCC	–	–	40–60 (usually related to lung carcinoma)	1:1
Acquired peripheral nerve Hyper excitability syndromes	Presynaptic	VGKC	–	–	Unknown	Unknown
Guillain–Barre syndrome	Presynaptic	GM1, GQ1b	–	–	Bimodal (adult and childhood)	1:1.25
Botulism	Presynaptic	–	BoNT toxin and SNARE proteins	–	Neonates and all other ages affected	–
Organophosphate Poisoning	Synaptic deft	–	Acetylcholinesterase	–	–	–
CMS	Presynaptic, synaptic deft or postsynaptic	–	Various mutated proteins	–	Predominanantly in childhood	–

other synapses and because of the availability of a highly abundant source of its close analog within the electric organs of electric fish. The information on the biology of the NMJ has assisted in the analysis of a series of diseases that affect its function resulting in motor weakness. The NMJ begins to form when the axon growth cone of a developing motor neuron, or a sprouting motor axon, encounters a developing myotube, or a denervated muscle fiber, and begins to secrete agrin, a glycoprotein with a laminin-binding domain that anchors it to the extracellular matrix [8–11]. Agrin can initiate the formation of the NMJ but requires the presence of the postsynaptic transmembrane kinase, muscle-specific kinase (MuSK). The latter is a receptor tyrosine kinase that, when activated by agrin, self-phosphorylates and phosphorylates a number of other proteins important to the formation of the NMJ, mediated through various downstream signal transduction pathways. The agrin/MuSK interaction requires mutual binding to a third transmembrane muscle protein, the low density lipoprotein receptor-related protein 4 (Lrp4) [12–15]. This process induces dense clustering of the AChRs in the postsynaptic membrane and marked folding and specialization of that membrane [8–11,16,17]. A number of less well understood processes also occur in the postsynaptic region, referred to as the muscle endplate (EP), that lead to: 1) secretion of acetylcholinesterase into the extracellular matrix, 2) concentration of sodium channels in the membrane of the valleys of the postsynaptic folds and 3) retrograde release of factors that induce the axon terminal to develop the specializations involved in activity-induced release of neurotransmitter (ACh)-containing synaptic vesicles. The mature NMJ (Fig. 1) consists of the specialized nerve terminal of the motor axon, which, when depolarized by an action potential, releases the ACh into the synaptic cleft. The released ACh diffuses across the cleft to bind to the very tightly packed AChRs located on the peaks of the highly folded EP membrane. The AChR is a multi-subunit transmembrane ligand-gated ion channel that opens upon binding of two molecules of ACh, resulting in cation influx and depolarization of the muscle membrane. When the depolarization reaches threshold, an action potential is initiated in the sodium channel-rich valleys of the synaptic folds leading to muscle contraction.

3. MG subgroups

Several subgroups of MG have been identified on the basis of clinical presentation, autoAb profile and thymic pathology including: early-onset (before age 40) MG, late-onset MG and thymoma associated-MG. Early-onset MG generally begins with ocular muscle weakness followed by generalized weakness and occurs more often in female patients. There is an association with HLA-B8DR3 and thymic hyperplasia [18]. In contrast, late-onset MG is more common in males over 60 years of age without thymoma [19]. There is no apparent gender predilection; however, compared to early-onset MG without thymoma, weakness including oropharyngeal involvement appears to be more severe and associated with additional autoAbs, including titin Abs [20], paraneoplastic Abs, voltage-gated K+ and Ca2+ channel Abs, Hu Abs, DHP related protein 5 and GAD Abs [21].

4. Role of the thymus in MG

Although the factors involved in the induction of autoimmune MG have not been fully elucidated, the association of MG with abnormalities of the thymus was described by Weingart as early as 1901. Approximately 10% of patients with autoimmune MG have a thymoma, which may possibly play a role in disease initiation through multiple mechanisms including the expression of self-antigens by thymoma cells and impaired negative selection of autoreactive T lymphocytes. Another 60% of patients have thymic hyperplasia, defined by the presence of medullary lymphoid follicles and germinal centers. While a body of evidence exists [22] that thymectomy produces long-term benefit in MG, the first randomized controlled clinical trial of this treatment is currently in progress.

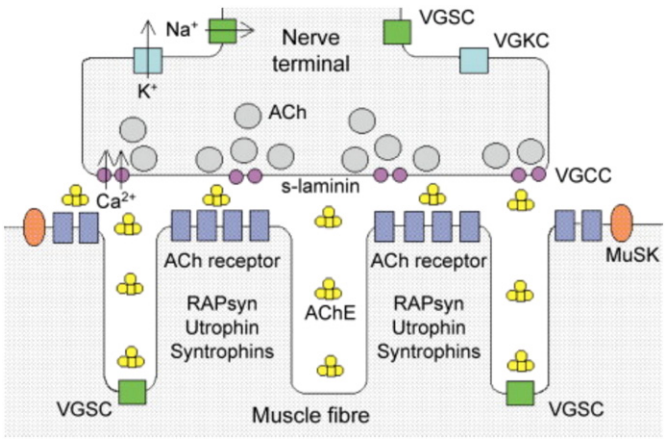

Fig. 1. Schematic of the neuromuscular Junction. VGSC = voltage-gated Na channel, VGKC = voltage-gated potassium channel, VGCC = voltage-gated calcium channel, AChE = acetylcholinesterase. Adapted from Vincent A, Newland C, Croxen R, Beeson D. Genes at the junction—candidates for congenital myasthenic syndromes. Trends Neurosci 1997; 20(1):15–22. Adapted with permission.

5. Anti-acetylcholine receptor antibody myasthenia gravis (AChR-MG)

Approximately ninety percent of patients with generalized acquired myasthenia have measurable AChR Abs circulating in blood (usually requiring the use of human AChR for the assay). For many years MG had been suspected to be an autoimmune disorder of the NMJ. Strong support of this hypothesis, as well as the identification of the autoantigen, derived from the serendipitous observation by Patrick and Lindstrom in 1973 that rabbits immunized, in order to obtain Abs, with AChR recently purified from fish electric organ, developed weakness and the electrophysiologic abnormalities similar to those in human MG [23]. This experimental disorder in rabbits, subsequently named experimental autoimmune myasthenia gravis (EAMG), was reproduced in other species, especially inbred Lewis rats [24] and also, to a lesser degree, susceptible strains of mice [25]. Clinical, pharmacological, histological, electrophysiological and immunological analysis of EAMG led to extensive observations related to the pathogenic mechanisms in this disease, [26,27] most of which have been found to hold for the human disease as well [26]. Acutely there is inflammatory destruction of the endplate membrane, whereas chronically the membrane appears to reform in a simplified fashion with reduced amounts of AChR [28].

AutoAbs and autoreactive T cells directed against the NMJ AChR are present in both MG and EAMG. However, the effects at the NMJ are carried out solely by the Abs, which primarily target the alpha 67-alpha76 portion of the alpha subunit of the AChR, known as the main immunogenic region (MIR) [29]. The Abs are primarily of the IgG1 and IgG3 isotypes and are thus capable of activating complement [30,31]. Three effector mechanisms of these Abs have been identified that result in clinical symptoms including 1) direct blockade of ACh binding sites inhibiting the opening of the ion channel, [32] 2) cross-linking of AChRs by divalent Abs accelerating endocytosis and degradation of AChRs, referred to as antigenic modulation [33,34] and 3) complement-mediated damage to the entire muscle endplate via formation of membrane attack complexes [35–37]. While direct binding of the AChR by Abs in mechanism 1 appears to play a minor role in pathogenesis, the second and third mechanisms result in removal of AChRs leading to clinically observed muscle weakness. In addition, the third mechanism, complement-mediated endplate membrane lysis, also destroys AChR-associated proteins required for NMJ function and maintenance.

6. MuSK antibody MG (MuSK-MG)

Patients who lack circulating Abs to AChR are referred to as having "seronegative MG" and have clinical characteristics that are similar, but not necessarily identical, to those with AChR Abs, i.e., seropositive MG. Seronegative patients were reported initially to respond as a group equally well to the treatments that are effective in seropositive patients [38]. Early studies demonstrated that IgM from some seronegative MG patients decreased AChR function in cultured muscle cell lines, but did not appear to bind directly to AChR [12,15,39]. Vincent et al. were able to identify an IgG in some patients that bound to an antigen on muscle cell lines that is distinct from AChR [12]. Further analysis identified the target as MuSK. These investigators went on to detect MuSK Abs in seventy percent of seronegative patients [14]. Larger studies with a more precise assay have determined that the figure is closer to 40 percent of seronegative patients and, to date, such Abs have been found only in patients with fatigable weakness [13,40–42]. This MuSK Ab-positive subgroup of seronegative patients has many clinical similarities to AChR MG, but tends to differ significantly in demonstrating more focal involvement than seropositive MG, frequently with severe involvement of neck, shoulder, facial and bulbar muscles, at times with wasting of these muscles, although there is considerable variability from patient to patient [40–43]. Also, no MuSK MG patient has been found to have thymic lymphoid hyperplasia (commonly found in MG) [44,45]. Very preliminary data suggest other differences, including observations that MuSK Ab patients respond especially well to plasma exchange, more so than to intravenous immunoglobulin [40], and do not respond to thymectomy [13,40,41]. Additionally, acetylcholinesterase inhibitors tend to be less effective and may even be counterproductive [40,41] while Rituximab appears to be more beneficial than in patients with AChR-MG [46].

MuSK is a 100 kD typical transmembrane receptor tyrosine kinase with an N-terminal extracellular domain followed by a short transmembrane domain and then a C-terminal cytoplasmic domain [47–50] (Fig. 2). This molecule is crucial to the interaction of muscle with agrin. MuSK responds by phosphorylating and binding a number of EP proteins, most importantly rapsyn, an intracellular protein that binds the intracellular domain of AChR and is likely the motor for dense AChR clustering [11,51,52]. The extracellular domain of MuSK, which appears to be required for interaction with agrin, comprises four immunoglobulin (Ig)-like domains. Between Ig-3 and Ig-4 is a cysteine-rich (frizzled-like) C6 box region [47–49]. The cytoplasmic domain contains the kinase activity and signaling components of the molecule leading to the development of the postsynaptic apparatus although binding to rapsyn appears to require a contribution from the Ig-4 region [53]. Studies employing both rat and human MuSK have determined that it is only the extracellular domain of the molecule that is the target of the MuSK-MG Abs [14,54]. These Abs induced in animals by active or passive immunization produce EAMG that mimics the human disease [55–58].

MuSK Abs are predominantly of the IgG4 isotype which is unable to bind complement factor C1q and thus does not cause complement activation [59]. Additionally, IgG4 undergoes a post-translation modification known as "Fab-arm exchange" which involves recombining half-antibodies with other IgG4 molecules, making them incapable of cross-linking antigens [60].

7. Lrp4 antibody MG (Lrp4-MG)

Lrp4 has been identified as a crucial postsynaptic protein for the development and maintenance of the neuromuscular junction [61] with specific function as the agrin receptor necessary for the activation MuSK [62,63]. Lrp4 Abs were hypothesized as a pathogenic factor in seronegative MG due to its postsynaptic localization and observations in Lrp4 null mutant animal models which showed similar phenotype to that observed in MuSK negative animals. Recent studies have shown antisera from double-seronegative (negative for AChR Abs and negative for MuSK Abs) MG patients specifically bound to HEK293 cells transfected with human Lrp4 and inhibited binding to agrin [64,65]. The Abs were found to be predominantly of the IgG1 isotype, which is capable of activating complement and thus producing damage to postsynaptic membrane folds via the membrane attack complex. Additionally, inhibition of agrin-induced aggregation of AChRs at the neuromuscular endplate has been implicated as a pathogenic mechanism in Lrp4-MG.

8. Lambert–Eaton myasthenic syndrome (LEMS)

LEMS is an autoimmune disorder affecting presynaptic P/Q-type voltage-gated calcium channels of the neuromuscular junction causing impaired quantal release of acetylcholine leading to muscle weakness. The P/Q-type VGCC is the basis for the action potential-induced Ca^{++} influx, which triggers fusion of the ACh-containing synaptic vesicles with the nerve terminal plasma membrane resulting in release of ACh into the synaptic cleft. Anderson in 1953 first described a patient with muscle weakness and diminished deep tendon reflexes with improvement in these symptoms after removal of a small-cell lung cancer [66]. A few years later Eaton and Lambert identified a series of similar cases with a distinctive incremental response on repetitive nerve stimulation [67]. Small-cell lung cancer and autonomic symptoms are common in LEMS [67,68]. Serum Abs to P/Q-type VGCC, which are present on

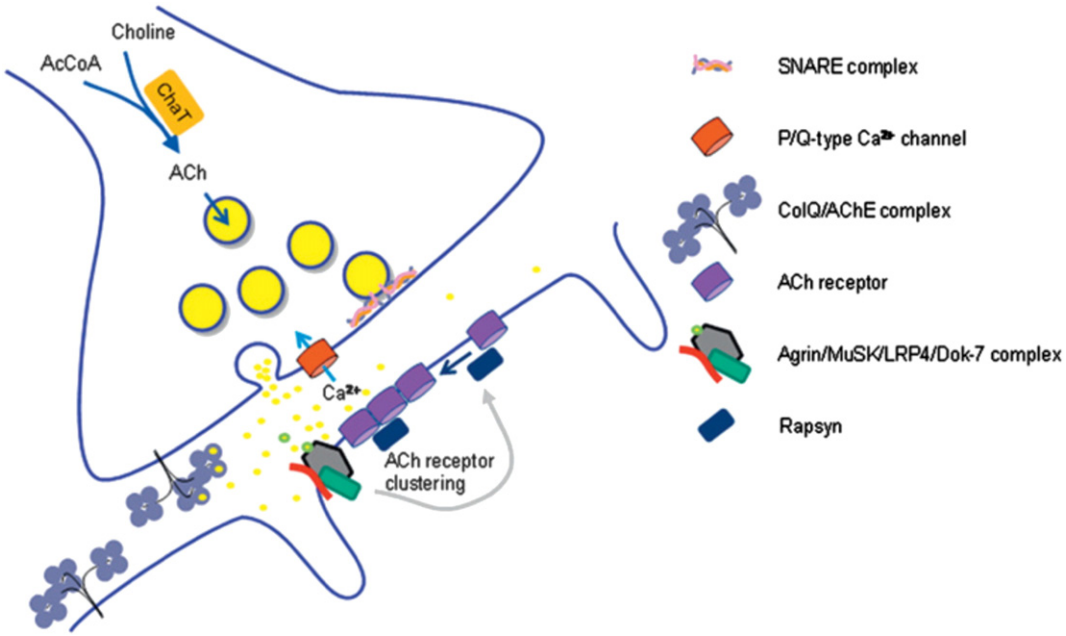

Fig. 2. Schematic of MuSK and associated molecules responsible for acetylcholine receptor clustering. AChE, acetylcholinesterase; AcCoA, acetyl coenzyme A; ChAT, choline acetyltransferase; ColQ, AChE collagen-like tail subunit; Dok-7, downstream of tyrosine kinase 7; LRP4, low-density lipoprotein receptor-related protein 4; MuSK, muscle-specific kinase; SNARE, soluble NSF attachment protein receptor. Adapted from Spillane J, Beeson D, Kullmann D. Myasthenia and related disorders of the neuromuscular junction. J Neurol Nurosurg Psychiatry 2010; 81:850–857. Adapted with permission.

small cell lung cancer cells and at motor nerve terminals, were detected by radioimmunoassay in 1995 [69,70]. These Abs are felt to be paraneoplastic in nature and directed at the small-cell lung cancer in a significant number of cases [69,71], though associations with other cancers including non-small-cell lung cancer, mixed lung carcinomas, prostate carcinoma, thymoma and lymphoproliferative disorders have been described [72,73]. The VGCC, like the AChR, is a complex protein made up of multiple subunits. ELISA and western blotting studies on the extracellular alpha1 subunit which is responsible for its pore forming properties have identified Abs to linker domains II and IV in approximately 50% of patients with LEMS. Although the antigenic trigger for the production of anti-VGCC Abs in patients without cancer is unknown, these cases have been found to have an association with the HLA haplotype B8 Dr2 DQ2 and other autoimmune diseases [74].

9. Acquired peripheral nerve hyperexcitability syndromes

The acquired peripheral nerve hyperexcitability syndromes include autoimmune neuromyotonia and cramp-fasciculation syndrome characterized by muscle cramps, weakness, fasciculations and increased sweating. Approximately 40% of cases have been associated with anti-voltage gated potassium channel Abs, which are felt to be the main pathogenic agents. Approximately 20% of cases have been associated with thymoma [6,75].

10. Guillain Barré syndrome

Guillain Barré syndrome and variants such as Miller Fisher syndrome are acute inflammatory autoimmune neuropathies which lead to demyelination and axonal damage. Infection by campylobacter jejuni and other pathogens preceding the onset of symptoms is common. Molecular mimicry between the infectious agents and ganglioside antigens on neural cells is felt to produce a cross-reactive humoral and cell mediated immune response. Approximately 20–60% of patient with Guillain Barré syndrome and Miller Fisher syndrome have Abs to GM1 and GQ1b, which have been shown to bind to gangliosides expressed in the NMJ and cause complement mediated changes in neurotransmitter release via disruption of the voltage-gated sodium channels [76–78].

11. Botulism

The anaerobic bacteria clostridium botulinum produces seven distinct neurotoxins, BoNT-A through BoNT-G, each of which disrupts neurotransmitter release (Fig. 3). The BoNT toxins are initially synthesized as an inactive single-chain protein and post-translationally modified into the active dichain molecule organized into a heavy and light chain linked by a disulfide bond [79,80]. The heavy chains contain binding and translocation functional domains while the light chains contain a catalytic zinc-endopeptidase domain which is specific for one of the following proteins of the neurotransmitter release apparatus: VAMP/synaptobrevin, SNAP-24 and syntaxin 1, the latter two proteins make up the SNARE (soluble NSF (N-ethylmaleimide-sensitive fusion protein) family of proteins involved in fusion of synaptic vesicles. A three step process has been described involving internalization of the BoNT at the presynaptic nerve terminal by receptor mediated endocytosis [81] followed by structural changes in the translocation domain which allow it to form a pore in the endosome membrane induced by the acidic pH of the endosome. The catalytic domain is then able to translocate across the endosome membrane and cleave one of the SNARE proteins, which prevents neurotransmitter release into the NMJ [82–84].

12. Organophosphate poisoning

Organophosphate compounds are used widely around the globe as insecticides and as “nerve agents” in chemical warfare. Exposure to these compounds leads to irreversible inhibition of acetylcholinesterase. Several syndromes associated with organophosphate poisoning have been described based on the timing of symptom onset including acute cholinergic crisis, intermediate syndrome, and organophosphate-induced delayed neuropathy (OPIDN). Organophosphate compounds were initially reported to inhibit AChE by Gross et al. in the 1930s [85]. Subsequent studies using diisoprophylflourophosphate in horse serum by Janz et al. showed that the mechanism involved the irreversible phosphorylation of a serine residue at the hydroxyl group in the active site of

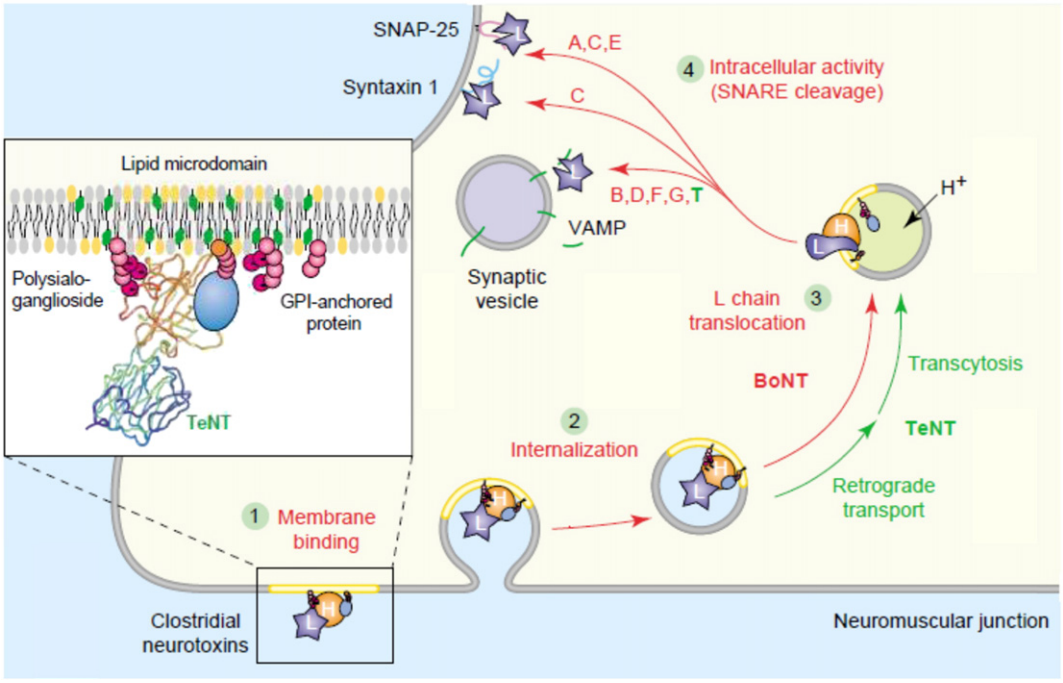

Fig. 3. Schematic of the four step mechanism of action of clostridial neurotoxins including (1) membrane binding, (2) internalization, (3) translocation and (4) intracellular action. SNAP-25, synaptosomal-associated protein 25; VAMP, vesicle associated membrane protein; SNARE, soluble NSF attachment protein; BoNT, botulinum neurotoxin; TeNT, tetanus neurotoxin. Adapted from Lalli G, Bohnert S, Deinhardt K, Verasteugui C, Schiavo G. The journey of tetanus and botulinum neurotoxins in neurons. Trends in Microbiology, 2003; 11(9)431–437. Adapted with permission.

acetylcholinesterase leading to irreversible AChE inhibition [86]. Although the mechanism has not been fully elucidated, this reaction leads to the prolonged accumulation of excess ACh at the NMJ and is felt to be the cause of failure of neuromuscular transmission.

13. Congenital myasthenic syndromes (CMS)

The CMS are a group of genetically determined diseases resulting from mutations in genes encoding proteins that are important for neuromuscular transmission (Fig. 4). These disorders are characterized by weakness and muscle fatigability that usually starts during the perinatal period or infancy. However, symptoms can start at any time in life posing diagnostic difficulties with seronegative myasthenia gravis. The first CMS thoroughly characterized was AChE deficiency by Andrew Engel in 1977 [87]. Electrophysiological and electron microscopic studies of intercostal and anconeus muscle biopsies were utilized to identify pre-synaptic vs. post-synaptic mechanisms by which various mutations caused CMS [87,88]. Since that time, molecular genetics through the use of the candidate gene approach, linkage analysis and whole exome sequencing has allowed the identification of an ever growing number of disease genes, though a large proportion remain unidentified [89,90]. Approximately 85% of identified CMS involve postsynaptic mutations

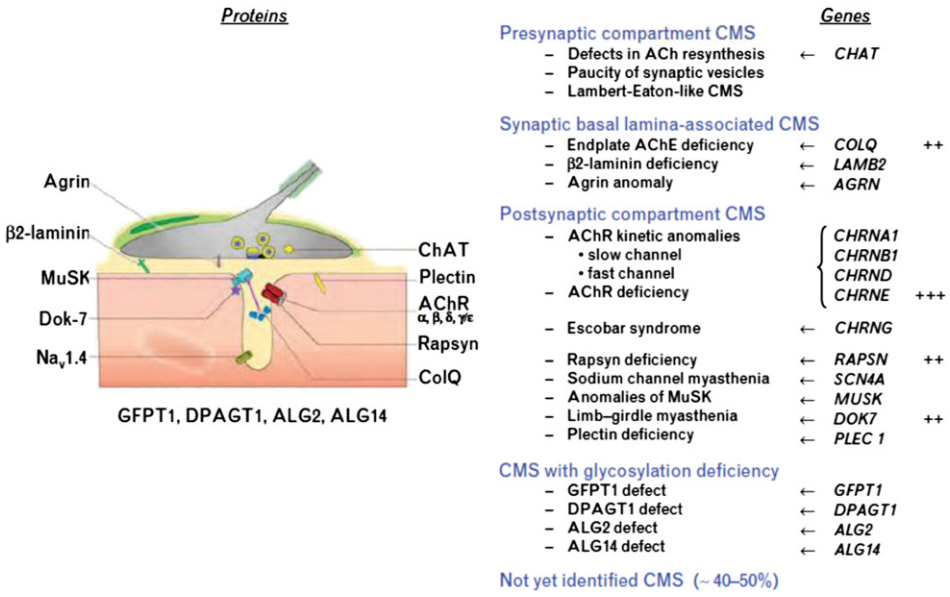

Fig. 4. Classification and localization of proteins encoded by genes associated with congenital myasthenic syndromes. Adapted from Hantaï D, Nicole S, Eymard B. Congenital Myasthenic syndromes: an update. Cur Opin Neurol. 2013; 26(5):561–568. Adapted with permission.

which can occur in any of the 4 subunits of the AChR as well as associated proteins including RAPSYN, SCN4A, MUSK, DOK7 and PLEC1. The most commonly mutated gene identified patients with CMS is the CHRNE, encoding the epsilon subunit of the AChR. Individual mutations in this gene can variously result in slow channel syndrome, fast channel syndrome or AChR deficiency [91,92]. AChR deficiency CMS is predominantly due to mutations located on the extracellular NH2 terminal region and in the M3/M4 loop due to missense, frameshfting, splice-site, nonsense and mico-deletions [92]. Other post-synaptic mutations have been identified in the CHRNA1, CHRNB1 and CHRND genes which can also cause kinetic anomalies including slow and fast channel CMS. Fast channel syndrome is caused by several different mechanisms including diminished affinity for acetylcholine, impaired gating efficiency and destabilization of channel kinetics [93]. Mutations involving the MuSK-Dok-7-Rapsyn pathway as well as SCN4A and PLEC1 genes have also been described which result in post-synaptic dysfunction of neuromuscular transmission [94]. Approximately 10% of CMS have been identified as synaptic basal lamina associated CMS such as in the COLQ gene mutation which results in endplate AChE deficiency, mutations in the LAMB2 gene which results in beta2-laminin deficiency and mutations in the AGRN gene which results in agrin anomalies. An estimated 5% of CMS are related to presynaptic defects including defects in the resynthesis of ACh due to mutations in choline acetyl-transferase (ChAT) and paucity of synaptic vesicles with yet unidentified genetic cause [95].

14. Conclusion

Diseases of the NMJ produce weakness through numerous mechanisms affecting the presynaptic, synaptic, and postsynaptic portions of the NMJ. Acquired, autoimmune Ab-mediated diseases have been identified and recently an ever growing number of genetic mutations have been identified which cause CMS. Advancements in elucidating the molecular and pathophysiological mechanisms underlying each disease has allowed for the development of treatment strategies specific for each pathogenic mechanism. In the future, advancements in molecular and genetic techniques may allow for greater understanding of NMJ disorders and even more effective and targeted therapies.

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